Methods of Optimizing Antibody Variable Region Binding Affinity

BACKGROUND OF THE INVENTION

This invention relates generally to a method of monoclonal antibody production and specifically to the simultaneous *in vitro* affinity optimization of multiple distinct domains of a variable region of a monoclonal antibody.

The War on Cancer is entering its third decade and recent years have shown tremendous progress in the understanding of cancer development and progression yet there has been only marginal decreases in death rates from most types of cancer. Standard chemotherapy and radiation therapy generally involve treatment with therapeutic agents that impact not only cancer cells but other highly proliferative cells of the body, often leading to debilitating side effects. Thus, it is desirable to identify therapeutic agents with a higher degree of specificity for the carcinogenic lesion.

The discovery of monoclonal antibodies (mAbs) in the 1970's provided great hope for the reality of creating therapeutic molecules with high specificity. Antibodies that bind to tumor antigens would provide specific targeting agents for cancer therapy. However, while the development of monoclonal antibodies has provided a valuable diagnostic reagent, certain limitations restrict their use as therapeutic entities.

A limitation encountered when attempts are made to use mAbs as therapeutic agents is that since mAbs are

developed in non-human species, usually mouse, they elicit an immune response in human patients. Chimeric antibodies join the variable region of the non-human species, which confers binding activity, to a human constant region. However, the chimeric antibody is often still immunogenic and it is therefore necessary to further modify the variable region.

One modification is the grafting of complementarity-determining regions, (CDRs) which are in part antigen binding onto a human antibody variable framework. However, this approach is imperfect because CDR grafting often diminishes the binding activity of the resulting humanized mAb. Attempts to regain binding activity require laborious, step-wise procedures which have been pursued essentially by a trial and error type of approach. For example, one difficulty in regaining binding affinity is because it is difficult to predict which framework residues serve a critical role in maintaining antigen binding affinity and specificity. Consequently, while antibody humanization methods that rely on structural and homology data are used, the complexity that arises from the large number of framework residues potentially involved in binding activity has prevented success.

Combinatorial methods have been applied to restore binding affinity, however, these methods require sequential rounds of mutagenesis and affinity selection that can both be laborious and unpredictable.

Thus, there exists a need for efficient and reliable methods for producing human monoclonal antibodies which exhibit comparable or enhanced binding affinities to their non-human counterparts. The present

invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides a method of conferring donor CDR binding affinity onto an antibody acceptor variable region framework. The method consists of: (a) constructing a population of altered antibody variable region encoding nucleic acids, said population comprising encoding nucleic acids for an acceptor variable region framework containing a plurality of different amino acids at one or more acceptor framework region amino acid positions and donor CDRs containing a plurality of different amino acids at one or more donor CDR amino acid positions; (b) expressing said population of altered variable region encoding nucleic acids, and (c) identifying one or more altered variable regions having binding affinity substantially the same or greater than the donor CDR variable region. The acceptor variable region framework can be a heavy or light chain variable region framework and the populations of heavy and light chain altered variable regions can be expressed alone to identify heavy or light chains having binding affinity substantially the same or greater than the donor CDR variable region. The populations of heavy and light chains additionally can be coexpressed to identify heteromeric altered variable region binding fragments. The invention also provides a method of simultaneously grafting and optimizing the binding affinity of a variable region binding fragment. The method consists of: (a) constructing a population of altered heavy chain variable region encoding nucleic acids comprising an acceptor variable region framework containing donor CDRs and a plurality of different amino acids at one or more